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Comparison Between Adsorption of Poliovirus and Rotavirus by Aluminum Hydroxide and Activated Sludge Flocs

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Adsorption of poliovirus and rotavirus by aluminum hydroxide and activated sludge flocs was studied. Both aluminum hydroxide and activated sludge flocs adsorbed greater amounts of poliovirus than rotavirus. Aluminum hydroxide flocs reduced the titer of poliovirus in tap water by 3 $\log_{10}$, but they only reduced the titer of a simian rotavirus (SA-11) in tap water by 1 $\log_{10}$ or less and did not noticeably reduce the number of human rotavirus particles present in a dilute stool suspension. Activated sludge flocs reduced the titer of added poliovirus by 0.7 to 1.8 $\log_{10}$ and reduced the titer of SA-11 by 0.5 $\log_{10}$ or less. These studies indicate that a basic difference in the adsorptive behavior of enteroviruses and rotaviruses exists and that water and wastewater treatment processes that are highly effective in removal of enteroviruses may not be as effective in removing other viral groups such as rotaviruses.

In addition to reducing the organic content of domestic and industrial wastes, wastewater treatment procedures such as the activated sludge process also reduce the number of viral pathogens present in wastewater (1, 11, 19, 21). Similarly, procedures such as alum flocculation and filtration that are designed to reduce the turbidity and color of tap water are also effective in removing viral particles (8, 13, 25). Bacterial viruses and poliovirus have been used in a number of studies to demonstrate the effectiveness of different water and wastewater treatment procedures in removing viruses (5, 12, 17, 20). In general, these studies have shown that both the activated sludge process and flocculation procedures, using metallic salts, are effective in reducing the number of bacterial viruses and polioviruses in tap water and wastewater. Flocculation with ferric sulfate and sedimentation proved consistently effective in removing viruses from water treated by a pilot water treatment plant (8).

Self-limited gastrointestinal illness of unknown etiology characterized by diarrhea, abdominal cramps, low-grade fever, and occasionally nausea and vomiting continues to be responsible for a large number of reported waterborne disease outbreaks in which sewage contamination of the water supply has been suspected. Between 1971 and 1974, 46% of all waterborne disease outbreaks reported in the United States were attributed to gastrointestinal illness of unknown etiology (6). Recently, a pathogen associated with infantile diarrhea has been found to be a 70-nm virus designated as duovirus, rotavirus, or reovirus-like (3, 4, 7a, 10). This virus appears to be a major cause of gastroenteritis in humans, and similar viruses have been implicated in gastrointestinal disease in other mammals (24). Recent evidence indicates that this virus can also cause gastroenteritis in adult humans (22). Large numbers of particles (10^9 per gram of feces) may be excreted by infected individuals (7). Since large numbers of viruses are present in the stools of infected individuals, contamination of water supplies appears possible. Therefore, the ability of certain water and wastewater treatment procedures to remove rotaviruses was evaluated. The ability of these processes to remove poliovirus was also determined as part of a comparative study since this virus has been used in other studies (5, 12). The agent associated with human infantile diarrhea cannot be cultivated routinely in cell culture at the present time and must be quantitated by electron microscopy or by immunological tests. However, the related simian rotavirus (SA-11) was available and was found to give easily recognizable cytopathic effects in primary baboon kidney cells. This virus is morphologically and antigenically related to the human agent (9, 18, 24). For these reasons, SA-11 was used as a model for human rotavirus in most experiments.

MATERIALS AND METHODS

Virus and viral assays. Plaque-purified poliovirus type 1 (strain LSc) was used in these tests. Samples were assayed after dilution in tris(hydroxymethyl)-

aminomethane-buffered saline containing 2% fetal calf serum. Plaque-forming units were determined as previously described (14), using the BGM cell line. A pool of stool suspensions containing human rotavirus was obtained from a previous study (15). Human rotavirus was detected by examination of samples negatively stained with phosphotungstic acid, using a pseudoreplica technique (15). Virus was scored as 4+ (>20 particles per field), 3+ (10 to 20 particles per field), 2+ (5 to 10 particles per field), or 1+ (0 to 5 particles per field). Simian rotavirus (SA-11) was kindly supplied by H. Malherbe and was grown in primary baboon kidney cells prepared as previously described (13). SA-11, diluted in the buffered saline described above but without fetal calf serum, was assayed by a quantal assay in tubes of baboon cells. The 50% tissue culture infectious dose was determined by the method of Reed and Muench (16).

Aluminum hydroxide flocs. A 0.01 M aluminum sulfate solution was neutralized by using 1 M sodium carbonate. The floc that formed at neutrality was collected by centrifugation and suspended in an equal volume of distilled water.

Activated sludge. Activated sludge was obtained from the aeration tanks of the Bellaire, Tex., sewage treatment plant. This sludge typically contained 5 mg of solids per ml (as determined by drying samples to constant weight at 105°C).

Experimental procedures. Poliovirus and SA-11 were added to tap water that had been dechlorinated by addition of 10 mg of sodium thiosulfate per liter. Suspensions of aluminum hydroxide flocs were added to 20 ml of the water at pH 7.2 to 7.8. The samples with floc and controls without floc were each mixed for 5 min and then centrifuged for 5 min at 2,000 × *g*. Virus remaining in the supernatants was determined and compared with the virus present immediately after addition of stock virus to the water samples. In a similar manner, aluminum hydroxide floc suspensions were added to 1 or 10% (vol/vol) stool suspensions containing human rotaviruses. In some cases the stool suspensions were mixed with a sample of poliovirus that had been concentrated as previously described (7a). After mixing and centrifuging as above, rotavirus in the supernatant was determined by electron microscopic observation of the samples, whereas poliovirus was measured as plaque-forming units. Poliovirus and SA-11 were added to fresh samples of activated sludge and to equal volumes of tap water. Virus in the water was assayed immediately, and the titer obtained was considered to be 100%. One sample of sludge was centrifuged immediately after addition of virus. Another sample of sludge and a tap water control were mixed for 20 min at 50 rpm on a rotary shaker and then centrifuged. Virus in the supernatants was determined.

RESULTS AND DISCUSSION

As part of a study on concentration of rotaviruses from stool suspensions, attempts were made to concentrate human rotavirus from stool suspensions by adsorbing the virus to aluminum hydroxide flocs. As shown in Table 1, little or no adsorption of the human rotavirus occurred. Since poliovirus will adsorb to aluminum hydroxide flocs under similar conditions, it was decided to compare adsorption of rotavirus and poliovirus in additional studies. Quantitation of the human rotavirus is difficult, and limited amounts of this virus were available. Therefore, the related simian rotavirus (SA-11) was used in additional studies. Adsorption of poliovirus and SA-11 by aluminum hydroxide flocs was compared, and the results are shown in Table 2. Whereas poliovirus was reduced by approximately 3 logs, SA-11 was reduced by 1 log or less. A difference in removal of the two viruses by activated sludge flocs was also observed, although sludge flocs adsorbed less of either virus than did the aluminum hydroxide flocs (Table 3). Activated sludge flocs reduced the titer of poliovirus by 0.7 to 1.8 logs, but only reduced the titer of SA-11 by 0.5 logs or less.

The results obtained with poliovirus are similar to those obtained in studies reported by other investigators (5, 12, 21). In general, poliovirus was readily adsorbed by activated sludge flocs and by aluminum hydroxide or other inor-

TABLE 1. *Removal of human rotavirus from stool suspensions by aluminum hydroxide flocs*[a]

Stool sample	Stool concn % (vol/vol)	Final concn of aluminum hydroxide floc (vol/vol)	Sample	Rotavirus	Poliovirus (PFU)[b]
A	10	1/10	Initial	++++	2.6×10^9
			Supernatant	++++	2.2×10^8
			Floc suspension	++	2.0×10^9
A	1	1/10	Initial	+	ND[c]
			Supernatant	++	ND
			Floc suspension	−	ND
B	10	1/40	Initial	++++	3.2×10^9
			Supernatant	+++	2.6×10^7

[a] A suspension of aluminum hydroxide floc was added to stool suspensions containing human rotavirus (with and without added poliovirus) to produce the indicated concentration of aluminum hydroxide floc (vol/vol). The samples were mixed for 5 min and centrifuged. Virus in the initial sample, in the supernatant remaining after centrifugation, and in a water suspension of the floc were determined.

[b] PFU, Plaque-forming units.

[c] ND, Not done.

TABLE 2. *Removal of poliovirus and SA-11 from tap water by aluminum hydroxide flocs*[a]

Trial	Sample	Log reduction	
		Poliovirus	SA-11
1	Tap water control	0.01	0.25
	Tap water + 1/10 floc	2.96	1.00
2	Tap water control	0.00	0.17
	Tap water + 1/20 floc	2.68	0.88
	Tap water + 1/40 floc	2.68	0.83
3	Tap water control	0.00	0.33
	Tap water + 1/20 floc	3.00	0.84
	Tap water + 1/40 floc	2.80	0.75

[a] Approximately 10^6 plaque-forming units of poliovirus or 10^6 50% tissue culture infectious dose of SA-11 were added to dechlorinated tap water. The samples were assayed immediately, and this value was taken as 100%. The indicated amount of floc was added, and the samples were mixed for 5 min and then centrifuged. Virus in the supernatants of samples with floc and controls without floc was determined and used to calculate reduction.

TABLE 3. *Removal of poliovirus and SA-11 by activated sludge flocs*[a]

Trial	Sample	Log reduction	
		Poliovirus	SA-11
1	Tap water control	0.01	0.00
	Activated sludge (initial)	0.36	0.00
	Activated sludge (final)	0.70	0.25
2	Tap water control	ND[b]	0.3
	Activated sludge (initial)	ND	0.2
	Activated sludge (final)	ND	0.3
3	Tap water control	0.1	0.3
	Activated sludge (initial)	1.1	0.5
	Activated sludge (final)	1.8	0.5

[a] Approximately 10^6 plaque-forming units of poliovirus or 10^6 50% tissue culture infectious dose of SA-11 were added to activated sludge and to equal volumes of dechlorinated tap water. Virus in the tap water was assayed immediately, and this value was taken as 100%. One sample of activated sludge was centrifuged immediately after addition of virus (initial), and the supernatant was assayed for virus. The other sample of sludge and a tap water control were mixed for 20 min and then centrifuged. Virus in the supernatants was determined (activated sludge, final sample).

[b] ND, Not done.

ganic floc. These works employing enteroviruses have been used to predict the amount of virus that will be removed by water and wastewater treatment processes (2, 11, 21). In light of the findings presented in this paper, the reported reductions of these viruses may not reflect the amount of rotavirus that is removed by unit treatment processes. This study suggests that the adsorptive characteristics of poliovirus and rotavirus are different, so that poliovirus cannot be used as a model to predict the removal of rotavirus. Based on the results obtained in this work, the expected removal of poliovirus and rotavirus by a two-stage treatment process is shown in Table 4. If equal numbers of both viruses are initially present, rotaviruses would predominate after treatment with activated sludge and aluminum hydroxide flocculation.

Ideally, the removal of viruses such as hepatitis A and human rotavirus should be studied by using these viruses. Since the virus associated with hepatitis A and human rotavirus cannot now be grown in cell culture, appropriate model viruses must be used to predict their behavior in water samples. It appears that SA-11 can be used as a model for human rotavirus. We have found that both SA-11 and human rotavirus adsorb to membrane filters at low pH and are eluted at high pH (7a; Farrah et al., unpublished data) and are not adsorbed to aluminum hydroxide flocs to the same extent as poliovirus. Other workers have observed a close morphological and antigenic relationship between human rotavirus and SA-11 (9, 18, 24).

The use of reovirus as a model for rotavirus in environmental studies should also be evaluated. These viruses are similar to rotaviruses in morphology and in their ability to be adsorbed by aluminum hydroxide flocs. Wallis and Melnick (23) observed that aluminum hydroxide flocs reduced the titer of poliovirus by 3.5 $\log_{10}$, but only reduced the titer of a human reovirus by 0.5 $\log_{10}$. The reduction in titer of reovirus by aluminum hydroxide flocs reported by these

TABLE 4. *Expected removal of rotavirus and poliovirus by treatment processes*

Sample	PFU[a] of:		Ratio of rotavirus/ poliovirus
	Poliovirus	Rotavirus	
Initial[b]	10,000	10,000	1
Supernatant after activated sludge treatment[c]	560	4,200	7.5
Supernatant after aluminum hydroxide flocculation and settling[d]	1	670	670

[a] PFU, Plaque-forming units.

[b] Assuming equal numbers of viruses are initially present per unit volume.

[c] Based on an expected 1.25 $\log_{10}$ reduction of poliovirus and an 0.38 $\log_{10}$ reduction of rotavirus (mean values from Table 3).

[d] Based on an expected 2.74 $\log_{10}$ reduction of poliovirus and an 0.79 $\log_{10}$ reduction of rotavirus (mean values from Table 2).

workers is similar to that for rotaviruses found in this study.

Though the use of model viruses can suggest the ability of treatment processes to remove rotaviruses from water and wastewater, procedures for the concentration and detection of human rotaviruses in water samples must be developed to determine the importance of this virus in the waterborne transmission of gastroenteritis.

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